



PATENT

IN THE UNITED STATES  
PATENT AND TRADEMARK OFFICE

APPLICANT(S): John Reynolds  
APPLICATION NO.: 10/009,389  
FILING DATE: 20 July 2002  
TITLE: Transformation of *Allium sp.* with *Agrobacterium* Using  
Embryogenic Callus Cultures  
EXAMINER: Georgia L. Helmer  
GROUP ART UNIT: 1638  
ATTY. DKT. NO.: 34703/0042

COMMISSIONER FOR PATENTS  
P.O. BOX 1450  
Alexandria, VA 22313-1450

**37 C.F.R. § 1.131 DECLARATION OF PRIOR INVENTION**

I, John Reynolds, hereby declare:

that I am the inventor named of the subject matter claimed in the above-identified patent application:

that I have reviewed the Office Action dated November 3, 2005, rejecting the patent application;

that I have reviewed the prior art references cited therein;

that the reference Eady, C.C., Transformation of Onion, 1998 Proceedings National Research Conference, Sacramento, CA, USA, was published in accordance with a conference that occurred between December 10 to December 12, 1998;

that the reference Eady, *et al.*, A comparison of four selective agents to use with *Allium cepa* immature embryos and immature embryo-derived cultures, Plant Cell Reports (1998) 18:117-121, published in the 1998 November issue of that publication;

that I have reviewed my records relating to the development of the subject matter claimed in the application, namely, methods for the transformation of *Allium sp.* with *Agrobacterium* using embryogenic callus cultures;

that subject matter reflected in the claims of the above patent application was invented by me, as reflected in my records, at least as early as October 1, 1998;

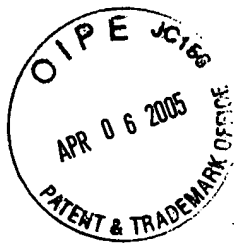
that the attached photocopies are from an internal Biotech Quarterly Report from Seminis Vegetable Seeds, Inc., from the 1st quarter of 1998 (which was produced by me prior to October 1, 1998), which has been redacted to remove confidential information not necessary to understanding the development of transformed *Allium cepa*;

that the Biotech Quarterly Report discloses results of experiments showing the development of transformed *Allium cepa* with a construct having as the DNA of interest a GUS reporter gene; and

that I, being warned that willful false statements and the like are punishable by fine or imprisonment, or both ( 18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon, declares that all statements made of the my own knowledge are true and that all statements made on information and belief are believed to be true.

Dated: 4.1.2005

By: John Reynolds  
John Reynolds, Inventor



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**37 C.F.R. § 1.132 DECLARATION TRAVERSING REJECTION**

I, John F. Reynolds, hereby declare:

that I am the inventor named of the subject matter claimed in the above-identified patent application:

that I have reviewed the Office Action dated November 3, 2005, rejecting the patent application;

that I have reviewed the prior art references and arguments cited therein;

that I have reviewed records relating to the development of the subject matter claimed in the application, namely, results of experiments relating to the transformation of *Allium sp.* with *Agrobacterium* using embryogenic callus cultures;

that the attached photocopy provides the results of a PCR experiment demonstrating the development of transformed *Allium cepa* lines in accordance with methods of the specification;

that the PCR experiment involved four transformed *Allium cepa* lines resistant to the herbicide glyphosate (lanes 2-5), one non-transformed *Allium cepa* line (lane 6), and a construct

transformed *Allium cepa* line (lane 6), and a construct having the heterologous 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene (lane 7), with lanes 1 and 8 being molecular weight markers;

that the transformed *Allium cepa* line was developed over the course of the last several years using techniques and starting materials set forth in the specification, namely, by a) culturing immature embryos from *Allium cepa* on an initiation medium for a period of from 2 to 6 months until an embryogenic callus formed, b) transferring the embryonic callus to a coculture medium and contacting the embryogenic callus with a suspension of *Agrobacterium tumefaciens* containing a DNA of interest from a heterologous gene, and c) obtaining a transformed *Allium cepa* embryogenic callus under selective conditions;

that the construct used in development of the transformed *Allium cepa* lines is the one described in Example 2 of the patent application, namely, pMON45312, that the DNA of interest was an EPSPS gene, and that the selective conditions used included growth of the transformed *Allium cepa* embryogenic callus on glyphosate;

that the PCR primers used in the PCR experiment were i) from the EPSPS gene, as a first set of PCR primers, and ii) from an onion seedling cDNA clone from GenBank number BE205672 submitted by J.A. McCallum et al. Theor. Appl. Genet. 103 (67), 979-991 (2001) used as a marker for onion genetic analysis, as a second set of PCR primers;

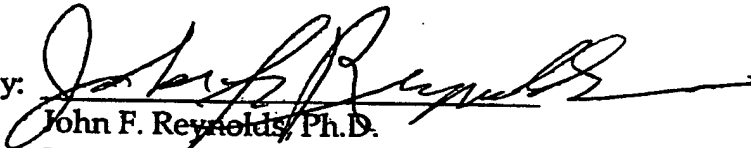
that the attached photocopy shows that the glyphosate resistant *Allium cepa* plants (lanes 2-5) each have been transformed to contain DNA of the heterologous EPSPS gene (lane 7), as evidenced by the common band generated by the first set of PCR primers;

that the attached photocopy shows that the control *Allium cepa* plant (lane 6) does not contain DNA of the heterologous EPSPS gene, but only DNA of the onion cDNA clone, as evidenced by the common band between all onion plants (lanes 2-6) generated by the second set of PCR primers; and

that I, being warned that willful false statements and the like are punishable by fine or imprisonment, or both ( 18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon, declares that all statements made of the my own knowledge are true and that all statements made on information and belief are believed to be true.

**PATENT**

Dated: 4.1.2005

By:   
John F. Reynolds, Ph.D.  
Inventor